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ORIGINAL PAPER

Comparative analysis of different groups of phenolic compounds in fruit and leaf extracts of *Aronia* sp.: *A. melanocarpa, A. arbutifolia*, and *A.* ×*prunifolia* and their antioxidant activities

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Abstract Anthocyanins, flavonols, and phenolic acids were estimated in methanolic extracts from the fruits and leaves of three chokeberries—*Aronia melanocarpa, Aronia arbutifolia*, and *Aronia*×*prunifolia*. The fruits contained significant amounts of cyanidin glycosides (0.3–323.2 mg/100 g DW) and two phenolic acids: chlorogenic acid (16.3–273.5 mg/100 g DW) and neochlorogenic acid (92.3–212.6 mg/100 g DW). The leaf extracts contained high amounts of flavonols: quercetin, quercitrin, and rutin (62.1–367.0 mg/100 g DW), as well as chlorogenic acid, neochlorogenic acid, and rosmarinic acid (max. 724.2, 482.7, 154.7 mg/100 g DW, respectively). Of the examined materials, *A. arbutifolia* leaves were characterized by the highest total phenolics content (9148.2 mg gal. ac. Eq./100 g DW) and showed the highest antioxidant activity in DPPH and FRAP assays. The results demonstrate that fruits of *A. arbutifolia* and *A.* ×*prunifolia* are a rich source of antioxidants and can be used as plant raw materials, alternatively to *A. melanocarpa* berries. Leaves of the investigated species are of potential therapeutic and dietary interest because of their high flavonol and phenolic acid content.

Keywords Anthocyanins · Flavonols · Phenolic acids · Antioxidant activity · Total phenols · Black chokeberry · Red chokeberry · Purple chokeberry

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Introduction

The fruit of *Aronia melanocarpa* (Michx.) Elliott (black chokeberry) is a well-known plant raw material used as a foodstuff, food supplement, and an ingredient in medicinal products and cosmetics. Numerous scientific studies have demonstrated antioxidant [1–3], anti-inflammatory [4], hepatoprotective, gastroprotective, UV protective [5], hypoglycemic, antimutagenic, and anticancer [6–9] properties of extracts from the fruits of black chokeberry. Scientific studies have also confirmed their beneficial effect on the cardiovascular system [2, 10, 11] and eye functioning [12]. The above-mentioned biological activities are attributed to phenolic compounds, mainly anthocyanins, flavonols, tannins, phenolic acids, organic acids, vitamins, and bioelements [13–16]. The fruit of *A. melanocarpa* proved to be extremely useful not only in phytotherapy, but also in

the food industry, especially as an ingredient of functional foods. Two other chokeberries, *Aronia arbutifolia* (L.) Pers. (red chokeberry) and *Aronia*×*prunifolia* (Marsh.) Rhed. (purple chokeberry), are less studied and hence little utilized by the pharmaceutical and agricultural crop industries [13].

All of three chokeberries analyzed in this study grow as shrubs in their natural habitats in North America. They are also successfully cultivated in Europe and Asia [13, 15, 17]. These chokeberries are similar in terms of growth habit, but some morphological features allow them to be distinguished. Ripe berries of *A. melanocarpa* are black, with a thick waxy coating. By comparison, the berries of *A.* ×*prunifolia* are purple black, while those of *A. arbutifolia* are smaller and bright red. Moreover, which is characteristic, the berries of *A. arbutifolia* remain durable in the winter time as they do not shrivel [13]. The chokeberry of particular interest is *A.* ×*prunifolia* which is a polyploid hybrid of *A. melanocarpa* and *A. arbutifolia*. It shows the intermediate morphological features between parent species, but is much closer to *A. melanocarpa* with almost the same blackish fruits and is often hardly determinable within the natural populations of that species. Furthermore, the individuals of *A.* ×*prunifolia* often show tendency to apomixis, which is also the reason for the high stability of this hybrid [18–20].

Aronia melanocarpa was originally exploited as a source of colorants for food and pharmaceutical industry and subsequently became the most popular and widely cultivated *Aronia* species [21]. *A. arbutifolia* and *A.* ×*prunifolia*, on the other hand, remained largely underutilized, and consequently, the reports on their chemical composition are scarce. Only a few studies demonstrated the presence of anthocyanins, phenolic acids, and flavonols in fruits of both plants [15, 19]. However, their polyphenol profile is not yet fully known and requires further studies [13].

The aim of this study was to comprehensively analyze, for the first time, the three chokeberry species: *A. melanocarpa, A. arbutifolia*, and *A.* ×*prunifolia* with respect to the most important groups of secondary metabolites they contain: anthocyanins, flavonols, and phenolic acids which are responsible for biological properties of aronia plants. The study involved mature fruits of arboretum-grown plants (black, purple, and red chokeberries, as well as fruits of *A. melanocarpa* used by some Polish herbal companies for the production of food supplements). Since leaves of several berry plants were demonstrated to contain substantial amounts of antioxidants for potential use in food and pharmaceutical industries [22], it was also decided to examine the leaves of the three *Aronia* species for the presence of aforementioned constituents. As harvest date was previously shown to affect secondary metabolite content of chokeberry leaves [23], these were collected at two fruit maturation stages. A comprehensive insight into the qualitative and quantitative profiles of the above-mentioned groups of metabolites in methanol extracts was achieved using the LC-DAD (flavonoids and phenolic acids) and LC-DAD-ESI-MS (anthocyanins) methods. In addition, a comparative assessment of antioxidant potential was conducted by determining the total polyphenol content using the Folin–Ciocalteu (FC) reagent, while FRAP and DPPH assays were performed to measure the antioxidant activity of extracts from the raw materials tested.

Materials and methods

Plant material

The plant material was harvested in 2013 in Rogów Arboretum—Warsaw University of Life Sciences, Forest Experimental Station in Rogów (Poland) (51°49′N, 19°53′E, ca. 190 m a.s.l.). The Arboretum is located in potential habitat of fertile deciduous forest, and potential natural vegetation is subcontinental oak-lime-hornbeam forest. The USDA Hardiness Zone is 6b, and the mean annual precipitation is 596 mm [24].

The plant material consisted of the leaves and fruits of the following representatives of *Aronia* genus: *A. melanocarpa* (Michx.) Elliott, *A. arbutifolia* (L.) Pers., and *A.* ×*prunifolia* (Marsh.) Rhed. The plants origin data are as follows: *A. melanocarpa*—single specimen, accession number 12535, germinated in 1988, from Kent County, Michigan, USA; *A. arbutifolia*—three specimens, accession number 12207, germinated in 1987, from Botanischer Garten Greifswald, Germany, materials from three specimens collected as bulk sample; and *A.* ×*prunifolia*—five specimens, accession number 15768, germinated in 2002, from Wayne County, Michigan, USA, 166 m a.s.l. 42°9′N, 83°16′W, materials from five specimens collected as bulk sample. The plants were taxonomically verified by scientific staff of Rogów Arboretum. Fruits and leaves were harvested separately in their maturity in September 2013. The phase of full ripeness of fruits has been estimated on the basis of the color and consistency of the fruits. Fruits of *A. arbutifolia* were collected as dark red and *A. melanocarpa* and *A.* ×*prunifolia* as black and purple black—in quite dark color. In addition, leaves were harvested in July when the fruits were immature (green and firm), but the leaves were in their best vegetative condition/time. The leaves harvested in July are designated in the present work as 'I', while those harvested in September as 'II'. All plant material was dried outside in the open air at 25 ± 2 °C for 10 days.

In addition, the study included dried, powdered fruits of *A. melanocarpa* received from three Polish herbal companies: company 'B'—the chokeberry fruits originated from

China, harvested in 2013; company 'C'—the fruits harvested from a Polish crop in Podlasie in 2012; and company 'D'—the fruits harvested from a Polish crop in Wielkopolska in 2012. The fruits were stored in refrigerators at 8–10 °C in plastic bags. This raw materials are used by the producers for direct sales or as a component of combined preparations and food supplements (e.g., tablets, capsules, herbal blends, and syrups). In the present work, the fruits of *A. melanocarpa* from Rogów Arboretum are referred to as *A. melanocarpa* 'A', while those from the herbal companies as 'B', 'C', and 'D', respectively. Moisture content of all the examined samples was determined (Binder FD Oven, forced convection, 105 °C, 3h) and included as Table S1 (Online Resource 1).

Extraction, DAD-LC and LC-DAD-ESI-MS analyses

Anthocyanins

Dried, powdered plant materials (fruits and leaves I and II), 0.5 g each (three replications), were extracted following the procedure described previously [25, 26], with slight modification. The samples were extracted at room temperature with acidified methanol (1 ml 30% HCl per 100 ml MeOH) using a magnetic stirrer (5 × 20 ml, 5 × 30 min, 300 rpm). The filtered extracts were pooled, concentrated in vacuo (type 350 rotary evaporator, Unipan, Poland), and made up to 10.0 ml with acidified methanol. Chromatographic separation was carried out in a reversed-phase mode, with gradient program adapted from the previous work [27]. Analyses were performed with the use of the Shimadzu system consisting of two solvent pumps LC-20AD, an autosampler SIL-20AC (8 °C), a diode array detector SPD-M20A, a mass spectrometry detector 2010EC, a column oven CTO-20AC (30 °C), and a DGU-20A3 degasser. Chromatographical analysis was performed on a Supelcosil LC-18 column (150 × 4.6 mm, 3 µm, Sigma-Aldrich Co.). The mobile phase consisted of A: 0.1% TFA in water and B: [acetonitrile/0.1% TFA] in water 50:50 v/v. The gradient elution was as follows: 0 min, 15% B; 60.00 min, 30% B; 80.00 min, 15% B; 85.00 min, and 15% B; 85.01 min, stop. The flow rate was 0.5 ml/min, and the injection volume was 20 µl. Mass spectrometric detection was performed in the positive ion mode (2 kV detector voltage) using selected ion monitoring (*m/z* 449, 419 and 287). The following parameters of electrospray ionization were applied: CDL (curved desolvation line) temp., 230 °C, heat block temp., 200 °C; nebulizing gas flow, 1.5 l/min. Quantification of anthocyanins performed with the use of external standard (cyanidin 3-*O*-glucoside, Extrasynthese) was based on the peak area at = 520 nm. Peaks were integrated by the LC–MS solution (ver. 3.40, Kyoto, Japan) software. Low (0.078–5.0 mg/l) and high (15–250 mg/l) concentration standard calibration curves were plotted using dilution series of cyanidin 3-*O*-glucoside.

Flavonoids and free phenolic acids

Dried, pulverized plant materials (fruits and leaves I and II) 0.5 g each, were extracted with methanol (50 ml) under reflux condenser for 2 h, to analyze free phenolic acids and flavonoid glycosides. In methanolic extracts, chromatographic quantification of estimated compounds was performed using a modified LC method [28, 29]. An LC-DAD system (Merck-Hitachi) and a Purospher RP-18e analytical column (4 × 250 mm, 5 ml; Merck) were used. The mobile phase consisted of: A—methanol: 0.5% acetic acid (1:4 v/v); B—methanol. A gradient program was as follows: 0–20 min, 0% B; 20–35 min, 0–20% B; 35–45 min, 20–30% B; 45–55 min, 30–40% B; 55–60 min, 40–50% B, 60–65 min, 50–75% B; and 65–70 min, 75–100% B, with a hold time of 15 min, at 25 °C. The flow rate was 1 ml/min, injection volume was 10 µl, and detection wavelength was set at 254 nm. Quantification was carried out by comparison with standards (UV-DAD spectra and t_r values) of the following phenolic acids: 3,4-dihydroxyphenylacetic, caffeic, chlorogenic, *o*-coumaric, *m*-coumaric, *p*-coumaric, ferulic, gallic, gentisic, hydrocaffeic, *p*-hydroxybenzoic, isoferulic, neochlorogenic, protocatechuic, rosmarinic, salicylic, sinapic, syringic, vanillic acids, and also precursor of one group of these compounds—cinnamic acid (Sigma-Aldrich Co.). Flavonoid standards included aglycones: kaempferol, luteolin, quercetin, and myricetin, as well as glycosides: apigetrin, cynaroside, hyperoside, quercitrin, rutin, trifolin, and witexin (all compounds from Sigma-Aldrich Co.). The representative chromatogram is shown in Fig. 1.

Antioxidant capacity

Samples preparation

Plant material samples—1 g each—were placed in the tube, and 5 ml of methanol solution (80 ml of methanol with 10 ml of 0.16 M HCl and 10 ml of distilled water) was added. In the next step, these samples were shaken for 1.5 h. After this time, the samples were centrifuged and the supernatant was collected. The obtained precipitates were subjected to a second dilution—5 ml of acetone (70 ml of acetone and 30 ml of distilled water). After 1.5 h of shaking, the samples were centrifuged (5000 r/min, 4500×*g*, MPW-55, Poland) and the supernatant was collected. The

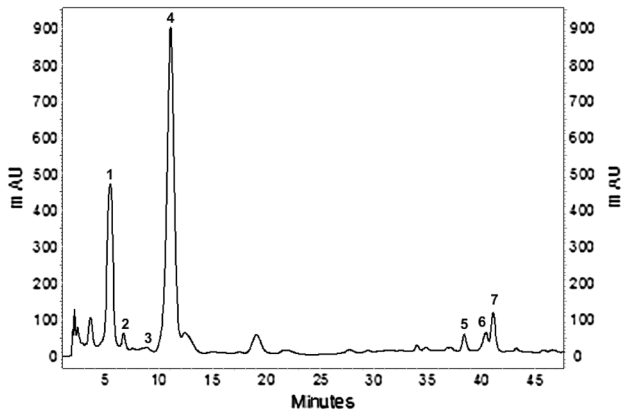

Fig. 1 LC-DAD chromatogram, separation of flavonols, and phenolic acids (example of separation of *A.* ×*prunifolia* leaves II extract); *1* neochlorogenic acid; *2* protocatechuic acid; *3* 3,4-dihydroxyphenylacetic acid; *4* chlorogenic acid; *5* rutin; *6* quercetin; *7* quercitrin

obtained supernatants (methanolic and acetonic) were mixed in a 1:1 ratio and used for antioxidant capacity analyses.

Total phenolics

The total polyphenols level was measured using the Folin–Ciocalteau reagent. The phenolic compounds present in the obtained extracts produced a blue color with the reagents used. All measurements were performed at a wavelength = 760 nm (JASCO C-530 spectrophotometer). Samples have been incubated for 30 min before measuring at temperature 25 ± 2 °C. As the standard, gallic acid was used in different concentrations: 0.00; 0.05; 0.15; 0.20; 0.25; and 0.3 g/l [30].

FRAP assay

The ferric ion reducing antioxidant parameter (FRAP) of the extracts was determined using the Benzie and Strain method [31]. Sample extracts diluted in methanol reduced Fe^{+3} to Fe^{+2} and produced a blue color with 2,4,6-tripyridyl-s-triazine (TPTZ) at a wavelength of 515 nm (JASCO C-530 spectrophotometer) at 37 °C. The results were calculated using the obtained standard curve (0.0; 0.1; 0.2; 0.3; 0.4; 0.5; 0.6; 0.8; and 1.0 mmol/L of Fe^{+2}).

DPPH assay

The inhibition of 1,1-diphenyl-2-picrylhydrazyl radical (DPPH) in analyzed samples was measured using the method by Brand-Williams et al. [32], with small modification. Absorptions for the samples diluted in methanol with DPPH solution were measured at 0, 15, and 30 min at a wavelength of = 515 nm (JASCO C-530 spectrophotometer), at 20 °C. Percentages of inhibition of the DPPH radical were calculated using the following formula: % of inhibition = $((Abs_0 - Abs_{15min})/Abs_0)*100\%$, where: Abs_0—absorption of DPPH solution before sample addition, and Abs_{15min}—absorption of DPPH solution after 15 min from sample addition.

Statistical analysis

Results were presented as mean ± standard deviation (SD) and were compared by the one-way analysis of variance (one-way ANOVA). For the comparison between different groups, the post-hoc Tukey HSD (honestly significant difference) test was used. STATISTICA version 12 PL software package (StatSoft) was used for the analysis. Values followed by the different letters in the same row are significantly different ($p < 0.05$). The letters correspond as follows: $^{a}p < 0.05$ vs. *A. melanocarpa* fruits A, $^{b}p < 0.05$ vs. *A. melanocarpa* fruits B, $^{c}p < 0.05$ vs. *A. melanocarpa* fruits C, $^{d}p < 0.05$ vs. *A. melanocarpa* fruits D, $^{e}p < 0.05$ vs. *A. arbutifolia* fruits, $^{f}p < 0.05$ vs. *A.* ×*prunifolia* fruits, $^{g}p < 0.05$ vs. *A. melanocarpa* leaves I, $^{h}p < 0.05$ vs. *A. melanocarpa* leaves II, $^{i}p < 0.05$ vs. *A. arbutifolia* leaves I, $^{j}p < 0.05$ vs. *A. arbutifolia* leaves II, $^{k}p < 0.05$ vs. *A.* ×*prunifolia* leaves I, $^{l}p < 0.05$ vs. *A.* ×*prunifolia* leaves II, $^{m}p < 0.05$ vs. all tested fruits and leaves samples, and $^{n}p < 0.05$ vs. all tested leaves samples.

Results

Analyses of anthocyanins

Fruits

The LC-DAD-ESI-MS analysis of extracts from the fruits of the studied *Aronia* species demonstrated the presence of cyanidin glycosides characteristic of *Aronia* plants [19]. The identification of anthocyanins was accomplished using the DAD detection and ESI-MS detection in the SIM mode: the presence of cyanidin 3-*O*-galactoside (Cy-Gal) and cyanidin 3-*O*-glucoside (Cy-Glu) was confirmed based on strong signals at *m*/*z* 449 (pseudomolecular ion) and *m*/*z* 287 (aglycone), whereas cyanidin 3-arabinoside (Cy-Ara) was detected by monitoring *m*/*z* 419 and *m*/*z* 287 ions [27, 33]. No cyanidin 3-*O*-xyloside was detected in any of the analyzed extracts. As presented in Fig. 2, the investigated compounds showed a typical anthocyanin elution pattern, with 3-*O*-galactoside (t_r = 35.5 min) followed by 3-*O*-glucoside (t_r = 40.0 min) and 3-*O*-arabinoside (t_r = 44.5 min) [34].

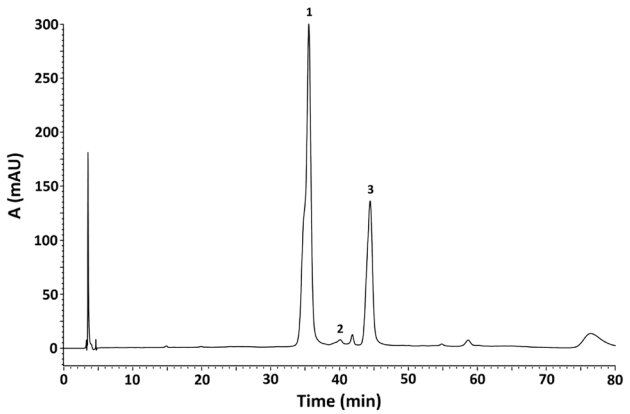

Fig. 2 LC-DAD-ESI-MS chromatogram, separation of anthocyanins (example of separation of *A.* ×*prunifolia* fruit extract); *1* Cy-Gal (cyanidin 3-*O*-galactoside); *2* Cy-Glu (cyanidin 3-*O*-glucoside); *3* Cy-Ara (cyanidin 3-*O*-arabinoside)

The highest total anthocyanin content was detected in the extract from *A.* ×*prunifolia* fruit (469.8 mg/100 g DW). In the extract from *A. melanocarpa* fruit, the total amount of anthocyanins was 283.5 mg/100 g DW. The lowest content was obtained in the extract from *A. arbutifolia* fruits—115.2 mg/100 g DW.

Based on the comparison of the amounts of anthocyanins in the fruit of *A. melanocarpa* of different origins, significant differences were found in total anthocyanin content in extracts from the fruit collected from the arboretum habitat (A)—283.5 mg/100 g DW—and from the fruit obtained from the pharmaceutical companies (B–D). The lowest total anthocyanin content was obtained in extracts from the fruit from the company B—26.9 mg/100 g DW—while the amounts of these compounds in the fruits from companies C and D were higher and almost the same—51.9 mg/100 g DW and 48.2 mg/100 g DW, respectively (Table 1).

The predominant compound in all the analyzed extracts from the fruit of *Aronia* sp. was Cy-Gal. The maximum amount of this compound was: in *A.* ×*prunifolia*—323.2 mg/100 g DW, in *A. melanocarpa*—210.8 mg/100 g DW, and in *A. arbutifolia*—104.7 mg/100 g DW. In the fruits of *A.* ×*prunifolia* and *A. melanocarpa*, there were also considerable amounts of Cy-Ara—140.9 mg/100 g DW and 61.8 mg/100 g DW, respectively. The amounts of Cy-Glu were markedly smaller, ranging from 0.3 to 10.8 mg/100 g DW (Table 1).

Table 1 Anthocyanin concentration (mg/100 g DW) measured in fruit and leaf tissue of three chokeberry species

Plant material	Cy-Ara	Cy-Gal	Cy-Glu	Total content
Fruits				
A. melanocarpa[x]				
A	61.8 ± 7.7^{m}	210.8 ± 33.1^{m}	10.8 ± 0.6^{m}	283.5 ± 41.5^{m}
B	7.2 ± 0.3^{af}	19.4 ± 0.6^{aef}	0.3 ± 0.1^{aef}	26.9 ± 0.9^{m}
C	14.1 ± 0.7^{af}	36.1 ± 1.8^{aef}	1.7 ± 0.1^{aef}	51.9 ± 2.6^{abef}
D	12.4 ± 1.1^{af}	34.4 ± 2.4^{aef}	1.4 ± 0.1^{aef}	48.2 ± 3.6^{abef}
A. arbutifolia	6.2 ± 0.6^{af}	104.7 ± 8.1^{m}	4.3 ± 0.4^{m}	115.2 ± 9.1^{m}
A. ×*prunifolia*	140.9 ± 12.9^{m}	323.2 ± 16.3^{m}	5.7 ± 0.7^{m}	469.8 ± 29.9^{m}
Leaves[y]				
A. melanocarpa				
I	nd	nd	nd	nd
II	0.2 ± 0.1^{m}	1.9 ± 0.1^{m}	nd	2.1 ± 1.0^{m}
A. arbutifolia				
I	nd	nd	nd	nd
II	nd	0.4 ± 0.1^{m}	nd	0.4 ± 0.1^{m}
A. ×*prunifolia*				
I	nd	0.2 ± 0.1^{m}	nd	0.2 ± 0.1^{m}
II	nd	1.2 ± 0.1^{m}	nd	1.2 ± 0.1^{m}

Data presented are the mean ± SD, $n = 3$

Cy-Ara cyanidin 3-arabinoside, *Cy-Gal* cyanidin 3-*O*-galactoside, *Cy-Glu* cyanidin 3-*O*-glucoside, *nd* not detected

[x]Fruits of *A. melanocarpa* of different origins: A—from arboretum habitat; B–D—fruits obtained from herbal companies

[y]I—leaves harvested in July, II—leaves harvested in September

Leaves

As compared to fruits, leaves of *Aronia* sp. were shown do contain low amounts of anthocyanins. Nevertheless, Cy-Gal was detected in all the extracts from leaves II (collected in September). The amounts of this compound did not exceed 2 mg/100 g DW, being equal to: in *A. melanocarpa*—1.9 mg/100 g DW, in *A.* ×*prunifolia*—1.2 mg/100 g DW, and in *A. arbutifolia*—0.4 mg/100 g DW. Leaves I (collected in July) of *A. melanocarpa* and *A. arbutifolia* did not contain anthocyanins. Cy-Gal was detected only in the leaves of *A.* ×*prunifolia* (0.2 mg/100 g DW).

Analyses of flavonols

Fruits

Of the 11 flavonoids (four aglycones and seven glycosides) estimated in extracts from the fruit of the studied *Aronia* sp.: *A. melanocarpa, A. arbutifolia*, and *A.* ×*prunifolia*, only one compound from the group of aglycones—quercetin was detected. The highest amount of quercetin was found in *A.* ×*prunifolia* fruit extracts—44.3 mg/100 g DW, followed by *A. arbutifolia* (31.8 mg/100 g DW) and *A. melanocarpa* (A) (12.2 mg/100 g DW, Table 2).

Among the fruits of *A. melanocarpa* of commercial origin, the highest amount of quercetin was found in the fruit obtained from the company B—24.9 mg/100 g DW. The amounts of quercetin in the fruit from the other companies were similar to those in the fruit from the arboretum (A), and were equal to 12.8 mg/100 g DW (C) and 15.9 mg/100 g DW (D) (Table 2).

Leaves

Both qualitative and quantitative differences were found between the studied species. Of the 11 flavonoids, the extracts from the leaves of *A. melanocarpa* and *A.* ×*prunifolia* were found to contain three compounds: one aglycone—quercetin and two glycosides—quercitrin and rutin. On the other hand, quercetin and its glycoside—quercitrin—were estimated in the leaves of *A. arbutifolia* (Table 2).

Differences between extracts from the leaves collected in July (I) and September (II) were also demonstrated. The highest flavonol content was found in the leaves of *A.* ×*prunifolia*. The total content in the leaves collected in July (786.4 mg/100 g DW) was higher than in those collected in September (614.4 mg/100 g DW). In the leaves of *A. melanocarpa*, the total amounts of flavonols were about two times lower than in *A.* ×*prunifolia* (284.5 mg/100 g DW for

Table 2 Flavonols concentration (mg/100 g DW) measured in fruit and leaf tissues of three chokeberry species

Plant material	Quercetin	Quercitrin	Rutin	Total content
Fruits				
A. melanocarpa[x]				
A	12.2 ± 0.6^{befn}	nd	nd	12.2 ± 0.6^{befn}
B	24.9 ± 0.8^{m}	nd	nd	24.9 ± 0.8^{acdfn}
C	12.8 ± 1.3^{bn}	nd	nd	12.8 ± 1.3^{befn}
D	15.9 ± 1.1^{bn}	nd	nd	15.9 ± 1.1^{befn}
A. arbutifolia	31.8 ± 3.3^{m}	nd	nd	31.8 ± 3.3^{acdefn}
A. ×*prunifolia*	44.3 ± 3.6^{m}	nd	nd	44.3 ± 3.6^{m}
Leaves[y]				
A. melanocarpa				
I	96.3 ± 2.5^{m}	111.9 ± 6.6^{n}	$76.3 \pm 3.0^{abcdefghijk}$	$284.5 \pm 12.2^{abcdefgijkl}$
II	108.8 ± 6.6^{m}	117.1 ± 2.6^{n}	62.1 ± 2.1^{n}	$288.1 \pm 11.3^{abcdefhijkl}$
A. arbutifolia				
I	83.2 ± 6.3^{m}	96.7 ± 5.4^{n}	nd	179.9 ± 11.7^{m}
II	94.8 ± 8.7^{m}	$165.1 \pm 14.5^{abcdefhijkl}$	nd	259.9 ± 23.2^{m}
A. ×*prunifolia*				
I	315.9 ± 21.3^{m}	367 ± 11.7^{n}	103.5 ± 3.7^{n}	786.4 ± 39.7^{m}
II	249.5 ± 12.5^{m}	289.9 ± 23.4^{n}	75.0 ± 3.3^{n}	614.4 ± 39.2^{m}

Data presented are the mean ± SD, $n = 3$

nd not detected

[x]Fruits of *A. melanocarpa* of different origins: A—from arboretum habitat; B–D—fruits obtained from herbal companies

[y]I—leaves harvested in July, II—leaves harvested in September

sample I and 288.1 mg/100 g DW for sample II). Likewise, in *A. arbutifolia*, the total flavonol content in the leaves collected in September (II) was higher—259.9 mg/100 g DW) than in the leaves from July (I)—179.9 mg/100 g DW (Table 2).

Analyses of phenolic acids

Fruits

Of the 20 compounds analyzed (19 phenolic acids and cinnamic acid—biogenetic precursor of one of group of phenolic acids), five were present in all the extracts from the fruits of the studied species of the genus *Aronia*: chlorogenic, 3,4-dihydroxyphenylacetic, neochlorogenic, protocatechuic, and rosmarinic acids.

The highest total amount of phenolic acids was confirmed in fruit extracts of *A.* ×*prunifolia*—503.9 mg/100 g DW, whereas *A. arbutifolia* fruits contained the lowest amounts (146.0 mg/100 g DW). In extracts from the fruit of *A. melanocarpa* harvested from the arboretum (A), the total amount of phenolic acids was higher in comparison with the fruits of commercial origin (B–D) (Table 3).

In fruit extracts of *A. melanocarpa* and *A.* ×*prunifolia*, two phenolic acids were dominant: chlorogenic acid (276.9 mg/100 g DW and 273.5 mg/100 g DW, respectively) and neochlorogenic acid (175.9 mg/100 g DW and 212.6 mg/100 g DW, respectively). By comparison, in the fruits of *A. arbutifolia*, a considerably higher amount of neochlorogenic acid was found (92.3 mg/100 g DW). The chlorogenic acid content was below 17 mg/100 g DW. In all the fruit extracts of the three *Aronia* sp., the amounts of the remaining phenolic acids ranged from 0.4 mg/100 g DW to 25.5 mg/100 g DW (Table 3).

Leaves

Of the 20 compounds included in the study (19 phenolic acids and cinnamic acid), the leaves of *A. melanocarpa* and *A.* ×*prunifolia* were found to contain four compounds: chlorogenic, 3,4-dihydroxyphenylacetic, neochlorogenic, and protocatechuic acids. In addition, rosmarinic acid was found in the leaves of *A. arbutifolia*.

In extracts from the leaves of *A. melanocarpa* and *A.* ×*prunifolia* collected in July (I), there were higher total amounts of phenolic acids (1191.8 mg/100 g DW and 1175.8 mg/100 g DW, respectively) than in those from the

Table 3 Phenolic acid concentration (mg/100 g DW) measured in fruit and leaf tissues of three chokeberry species

Plant material	Chlorogenic acid	3,4-Dihydroxy-phenylacetic acid	Neochlorogenic acid	Protocatechuic acid	Rosmarinic acid	Total content
Fruits						
A. melanocarpa[x]						
A	276.9 ± 19.2^{m}	9.9 ± 1.6^{m}	175.9 ± 3.6^{cdefn}	10.4 ± 0.8^{m}	14.4 ± 0.4^{bfn}	487.4 ± 25.6^{eghjkl}
B	151.2 ± 6.1^{m}	18.0 ± 1.4^{adefn}	175.2 ± 6.7^{cdefn}	30.9 ± 4.2^{m}	18.1 ± 2.7^{acdn}	393.4 ± 21.0^{eghjkl}
C	124.3 ± 4.0^{abefn}	17.8 ± 0.7^{adefn}	$144.4 \pm 7.9^{abcdefghjkl}$	13.4 ± 1.3^{m}	13.7 ± 1.5^{bfn}	313.6 ± 15.4^{fghjkl}
D	117.0 ± 5.1^{abefn}	25.5 ± 2.1^{m}	110.6 ± 5.6^{m}	19.3 ± 1.2^{m}	15.0 ± 1.7^{bn}	287.3 ± 15.8^{fghjkl}
A. arbutifolia	16.3 ± 0.5^{m}	20.8 ± 0.1^{m}	92.3 ± 3.3^{m}	0.4 ± 0.1^{m}	16.2 ± 1.3^{cn}	146.0 ± 5.4^{aben}
A. ×*prunifolia*	273.5 ± 4.7^{bcden}	4.3 ± 0.1^{m}	212.6 ± 1.6^{m}	$4.4 \pm 0.1^{abcdefghijl}$	9.2 ± 1.0^{m}	503.9 ± 7.5^{cden}
Leaves[y]						
A. melanocarpa						
I	705.8 ± 6.1^{m}	5.8 ± 0.3^{m}	473.0 ± 6.8^{m}	7.2 ± 1.1^{m}	nd	$1191.8 \pm 14.3^{abcdefghijl}$
II	426.4 ± 6.9^{m}	10.2 ± 0.6^{bcdefgij}	333.9 ± 6.3^{m}	$1.6 \pm 0.1^{abcdefghikl}$	nd	$772.1 \pm 13.9^{abcdefghijk}$
A. arbutifolia						
I	184 ± 12.7^{m}	38.0 ± 0.5^{m}	143.5 ± 1.4^{abdefn}	9.2 ± 0.4^{m}	23.3 ± 3.0^{m}	398.0 ± 18.0^{en}
II	724.2 ± 4.1^{m}	66.5 ± 1.7^{m}	450.2 ± 7.6^{m}	2.5 ± 0.4^{abcdefgijk}	154.7 ± 1.9^{m}	1398.1 ± 15.7^{m}
A. ×*prunifolia*						
I	678.2 ± 8.4^{m}	9.6 ± 0.4^{bcdefgij}	482.7 ± 4.9^{m}	5.3 ± 0.7^{abcden}	nd	$1175.8 \pm 14.3^{abcdefhijl}$
II	585.3 ± 6.1^{m}	9.3 ± 0.4^{bcdefgij}	353.9 ± 4.1^{m}	2.3 ± 0.1^{abcdefgik}	nd	$950.9 \pm 10.7^{abcdefgij}$

Data presented are the mean ± SD, $n = 3$

nd not detected

[x]Fruits of *A. melanocarpa* of different origins: A—from arboretum habitat; B–D—fruits obtained from herbal companies

[y]I—leaves harvested in July, II—leaves harvested in September

leaves collected in September (II) (772.1 mg/100 g DW and 950.9 mg/100 g DW, respectively). In *A. arbutifolia* leaf extracts, the total amount of phenolic acids was higher for the samples collected in September (1398.1 mg/100 g DW) than in July (398.0 mg/100 g DW) (Table 3).

The predominant compounds in all the leaf extracts were chlorogenic and neochlorogenic acids. The amounts of these compounds ranged from 184.0 to 678.2 mg/100 g DW and from 143.5 to 482.7 mg/100 g DW, respectively. In addition, in extracts from the leaves of *A. arbutifolia* collected in September, high amounts of 3,4-dihydroxyphenylacetic acid (66.5 mg/100 g DW) and rosmarinic acid (154.7 mg/100 g DW) were estimated (Table 3).

Antioxidant capacity

Fruits

Based on FRAP (mmql Fe^{+2}) and DPPH (% of inhibition) parameters, the strongest antioxidant activities were estimated for *A. arbutifolia* fruit extracts. The antioxidant activities of *A.* ×*prunifolia* and *A. melanocarpa* fruits were somewhat lower (Table 4).

The analyses of total phenolics contents estimated with Folin–Ciocalteu reagent showed similar amounts for the fruits of all the studied *Aronia* sp. Antioxidant parameters estimated for *A. melanocarpa* fruits of commercial origin (B–D) were lower than those estimated for the fruits collected from the natural habitat (A) (Table 4).

Leaves

The obtained results showed that the leaves of all the studied *Aronia* sp. possess strong antioxidant capacity. Extremely high DPPH and FRAP values were estimated for leaf extracts. In the initially prepared samples at 1100 times dilution, the DPPH (% of inhibition) and FRAP (mmql Fe^{+2}) values for the leaves of *A. arbutifolia* and *A. melanocarpa* collected in September (II) were higher than for the leaves collected in July (I). The estimated DPPH and FRAP values for the leaves of *A.* ×*prunifolia* were lower, and independent of the collection time (Table 4). Similar relationships were observed in the assays of total phenolics content estimated with the Folin–Ciocalteu reagent (Table 4). The highest polyphenol contents were estimated for the leaves of *A. arbutifolia* and *A. melanocarpa* collected in September (II), while values recorded for the

Table 4 Antioxidant parameters of extracts from fruit and leaf tissues of three chokeberries

Plant material	DPPH (% of inhibition ± SD) in 1100 times diluted samples		FRAP (mmpl Fe^{+2}/100 g DW ± SD)		Total Phenolics mg gal. ac. Eq./100 g DW ± SD
	15 min	30 min	15 min	30 min	
Fruits					
A. melanocarpa[x]					
A	31.5 ± 1.5^{bcdeghikl}	46.4 ± 3.5^{bcdfhijkl}	77.0 ± 3.6^{bcdfgijkl}	60.7 ± 3.1^{cen}	2815.3 ± 185.9^{m}
B	24.5 ± 1.4^{aefn}	27.3 ± 1.4^{aeghjkl}	53.2 ± 3.1^{acdegij}	62.7 ± 3.5^{cen}	2494.4 ± 113.6^{m}
C	23.1 ± 1.3^{aefn}	26.9 ± 1.4^{aeghjkl}	43.8 ± 2.0^{abefn}	52.1 ± 2.2^{aben}	$3009.0 \pm 94.9^{abcdefghijk}$
D	23.8 ± 1.1^{aefn}	27.7 ± 1.5^{acen}	44.9 ± 2.3^{aben}	54.5 ± 2.5^{en}	$2774.9 \pm 105.0^{abcden}$
A. arbutifolia	$41.4 \pm 1.8^{abcdefhijkl}$	46.4 ± 3.5^{bcdefn}	$77.0 \pm 3.1^{bcdefgijkl}$	$89.5 \pm 2.5^{abcdefijkl}$	3064.1 ± 187.7^{m}
A. ×*prunifolia*	28.4 ± 0.8^{m}	32.2 ± 1.6^{acen}	50.2 ± 1.3^{aceghij}	59.4 ± 1.7^{en}	$2746.8 \pm 177.8^{acbefn}$
Leaves[y]					
A. melanocarpa					
I	40.9 ± 1.5^{abcdfn}	50.7 ± 3.1^{bcden}	60.5 ± 4.8^{acdefghij}	86.0 ± 7.7^{abcdfn}	5005.5 ± 131.2^{m}
II	50.5 ± 2.0^{m}	61.7 ± 3.3^{m}	82.4 ± 2.1^{bcdkn}	95.7 ± 1.9^{abcdfn}	6892.6 ± 59.6^{m}
A. arbutifolia					
I	17.2 ± 1.7^{m}	24.2 ± 4.4^{aefn}	22.1 ± 0.1^{m}	33.2 ± 2.0^{m}	1946.8 ± 57.7^{m}
II	72.6 ± 1.9^{m}	92.1 ± 8.9^{m}	114.7 ± 9.2^{m}	145.7 ± 11.5^{m}	9148.2 ± 294.7^{m}
A. ×*prunifolia*					
I	$35.8 \pm 1.0^{abcdefghijk}$	$41.1 \pm 2.0^{abcdefghijk}$	58.5 ± 1.5^{acdegh}	$69.5 \pm 1.0^{abcdefghijk}$	2916.3 ± 190.6^{m}
II	$34.2 \pm 1.0^{bcdefghijl}$	$41.1 \pm 1.2^{abcdefghijl}$	57.9 ± 1.9^{acdegh}	$72.4 \pm 3.1^{abcdefghijl}$	3003.1 ± 77.3^{abdefn}

Data presented are the mean ± SD, $n = 3$

nd not detected

[x]Fruits of *A. melanocarpa* of different origins: A—from arboretum habitat; B–D—fruits obtained from herbal companies

[y]I—leaves harvested in July; II—leaves harvested in September

leaves of *A.* ×*prunifolia* were independent of the vegetation period.

Discussion

The presented work revealed differences in phenolic composition of fruits and leaves of the studied *Aronia* species (Figs. 3, 4). Moreover, significant differences were noted not only for the fruits of the different species of *Aronia* but also of the fruits of *A. melanocarpa* of different origins, as well as in the leaves of these species collected at different times. High antioxidant properties were demonstrated for all of the examined materials: in the case of fruits, it can be largely attributed to the presence of anthocyanins and phenolic acids, while in the leaves, the pivotal antioxidant role is played by flavonols and phenolic acids.

The highest total amount of anthocyanins was estimated in the fruit extracts of *A.* ×*prunifolia*, which is 1.7 times higher than in the fruits of the most common species—*A. melanocarpa*, and as much as 4.1-times higher than in the fruits of *A. arbutifolia* (Table 1; Fig. 3). The fruits of *A.* ×*prunifolia* were also found to have the highest amounts of the dominant cyanidin glycosides: Cy-Gal and Cy-Ara. Cy-Gal was the predominant color compound in all the studied chokeberries. The obtained results are consistent with earlier analyses of the chemical composition of *Aronia* sp. fruits [13, 15, 17, 19]. However, none of the fruits analyzed in the current study contained Cy-Xyl, which was reported by other teams [15, 19].

A. ×*prunifolia* is polyploid hybrid [17] which can partially explain its higher secondary metabolite content [35]. However, other reports do not indicate that anthocyanin content in this hybrid is necessarily higher in comparison with *A. melanocarpa* and *A. arbutifolia*. The study by Wangensteen et al. [25] confirms this observation, but the paper by Taheri et al. [13] does not.

The analyzed extracts from the fruits of *A. melanocarpa* obtained from different herbal companies (samples B-D) showed the same qualitative anthocyanins composition as extracts from the fruits collected from the natural habitat (sample A). However, noticeable differences in total anthocyanin content were observed, with fruits A containing ca. 5.5 times more anthocyanins than samples C and D, and over 10 times more than sample (B). Differences between accessions were also reported in other studies. For instance, Taheri et al. [13] recorded over sixfold difference in anthocyanin concentration between *A. melanocarpa* accessions, and ca. 2.5-fold difference for accessions of *A.* ×*prunifolia*, all grown in United States. In another study, Wu et al.

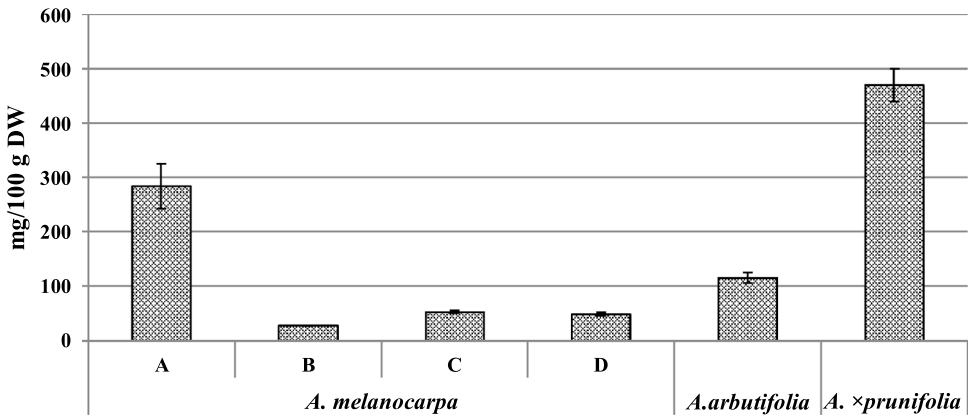

Fig. 3 Total anthocyanin concentration (mg/100 g DW ± SD, $n = 3$) estimated in fruits of studied chokeberry species (the fruits of *A. melanocarpa* of different origins: *A* from arboretum habitat; *B–D* fruits obtained from herbal companies)

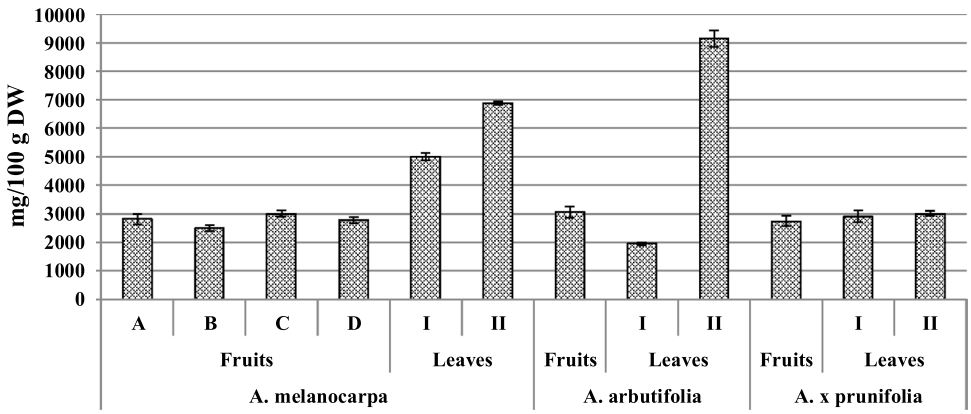

Fig. 4 Total flavonols and phenolic acids concentration (mg/100 g DW ± SD, $n = 3$) estimated in fruits and leaves of studied chokeberry species (the fruits of *A. melanocarpa* of different origins: *A* from arboretum habitat; *B–D* fruits obtained from herbal companies. The leaves: *I* leaves harvested in July; *II* leaves harvested in September)

[33] found nearly 1500 mg total anthocyanins per 100 g fresh aronia berries, that is ca. 3.7 times more than the lowest amounts estimated by Taheri et al. [13]. Differences between cultivars, albeit less prominent (ca. 1.5-fold), were reported by Jakobek et al. [36] (plants grown in Croatia) and Wangensteen et al. [25] (plants grown in Germany and Norway). Fruits of the same species/variety grown in different locations also differed with respect to anthocyanin content: there was ca. 1.3-fold difference between *A. melanocarpa* 'Nero' fruits harvested in Germany and Croatia [25, 36] and 2.0–4.7-fold difference between *A.* ×*prunifolia* grown in Germany and United States [13, 25]. Since factors such as maturation stage, fertilizing, and post-harvest procedures (e.g. drying) were shown to affect anthocyanin accumulation in aronia [15, 37–39], it is difficult to assess whether the above-mentioned differences result only from intra-species variation and climate conditions. Detailed cultivation conditions are often not included in the reports, making data interpretation harder. It is also worth noting that anthocyanin concentrations recorded in the present work (particularly those estimated in commercial samples) were noticeably lower in comparison with other studies (e.g. those by Wu et al. [33] and Taheri et al. [13]. These differences are likely due to air-drying of the samples employed in the current work. As indicated by literature data [39], chokeberry drying at 50–70 °C results in ca. 4.0-fold decrease in anthocyanin content as compared to fresh fruits. Freeze-drying, on the other hand, causes less than 2.0-fold decrease of anthocyanin concentration [39] which explains higher contents recorded in some other work [13, 33]. In our study, the concentrations estimated in fruits obtained from arboretum-grown plants are closer to the results by Ćujić et al. [40] who reported anthocyanin content of ca. 200–250 mg/100 g dry weigh in dried *Aronia* berries. Anthocyanin levels in fruits from different companies (samples B–D) were even lower, likely due to combined effect of different factors, such as the selection of low-yield accessions and the drying method applied. The practical conclusion is that batches of aronia fruits used in the production of natural drugs or diet supplements should be routinely screened for anthocyanin content. Moreover, standardized cultivation practices (growing conditions, application of fertilizers) and post-harvest treatments (water removal procedures and fruit storage) need to be employed to provide a high-quality market product.

The analysis of flavonols revealed no significant amounts of these metabolites in the fruits of the species studied. Of the compounds analyzed, only quercetin was found, with the highest amounts present in the fruit of *A.* ×*prunifolia* (Table 2; Fig. 4).

Regarding the analyses of phenolic acids in the fruits of *Aronia* sp., it is evident that the fruits of *A. melanocarpa* and *A.* ×*prunifolia* are a richer source compared to the fruit of *A. arbutifolia*, where the total amount of these compounds was about 3.4 times lower (Table 3 and Fig. 4).

The dominant phenolic acid in fruit extracts of *A. arbutifolia* was neochlorogenic acid (92.3 mg/100 g DW), whereas in the fruits of *A. melanocarpa* and *A.* ×*prunifolia* chlorogenic and neochlorogenic acid were detected in high quantities (276.9 mg chlorogenic acid and 175.9 neochlorogenic acid per 100 g DW in *A. melanocarpa*; 273.5 mg chlorogenic acid, and 212.6 mg neochlorogenic acid per 100 g DW in *A.* ×*prunifolia*). In other studies, these compounds have also been estimated in the fruit of *Aronia* sp. as the main representatives of this group of metabolites [13, 15, 17, 19], but other phenolic acids have not been detected previously. In the present work, phenolic acids such as 3,4-dihydroxyphenylacetic, protocatechuic, and rosmarinic acid, were determined in fruit extracts of *Aronia* sp. for the first time. Nevertheless, it has to be noticed that these compounds were only tentatively identified by LC-DAD method. Further analyses are required in order to unambiguously identify the above-mentioned metabolites.

The fruit extracts from *A. melanocarpa* of commercial origin were found to have similar qualitative composition of phenolic acids; however, they were estimated in lower quantities in comparison with naturally growing arboretum's plants.

The analysis of different groups of phenolic compounds in the leaves of the studied *Aronia* species showed high levels of phenolic acids and flavonols. Considerable qualitative and quantitative differences were revealed which depended not only on the species but also on the time of harvesting of the raw material.

The analysis of flavonols showed that the leaves of *A.* ×*prunifolia* collected in July had higher flavonol content (786.42 mg/100 g DW) in comparison with the two other plants (Table 2). Extracts from the leaves of *A.* ×*prunifolia* and *A. melanocarpa* were found to contain three flavonols: quercetin, quercitrin, and rutin. Leaves of *A. arbutifolia* were shown to be the poorest source of these compounds. Other research groups obtained different results when analysing flavonoids in the leaves of *A. melanocarpa*. Thi and Hwang [1] estimated only one compound—rutin, whereas the work by Lee et al. [23] revealed a rich composition of flavonoids, consisting mainly of various glycosides of apigenin, isorhamnetin, kaempferol, and quercetin. Higher amounts of these compounds were estimated in younger leaves, harvested in Korea in July.

The dominant compounds in all the analyzed leaves of the studied *Aronia* sp. were phenolic acids. In the leaves of *A. melanocarpa* and *A.* ×*prunifolia*, higher total amounts of phenolic acids (1191.8 mg/100 g DW and 1175.8 mg/100 g DW, respectively) were obtained in extracts from the leaves collected in July (I) when fruits were immature. This observation is in agreement with the previous study by Thi and

Hwang [1] and Lee et al. [23] who reported higher phenolic acid content in younger *A. melanocarpa* leaves. On the other hand, leaves of *A. arbutifolia* collected in September (II) had higher phenolic acid content (1398.1 mg/100 g DW, Table 3; Fig. 4) than leaves harvested in July. This phenomenon (as well as observed differences in flavonoid content) is likely species-specific and may result from different accumulation profiles of phenolic compounds in the investigated chokeberry species. As demonstrated in other plants [41], the accumulation of flavonoids and phenolic acids in leaves during vegetative cycle shows distinct maxima which can be expected to differ between species. However, determining whether such differences are also within *Aronia* genus would require further studies, involving harvesting of leaves at shorter intervals during vegetative period.

The leaves of *A. melanocarpa* and *A. ×prunifolia* were found to have a similar qualitative composition of phenolic acids. Four compounds were detected: chlorogenic and neochlorogenic acids (major constituents), as well as 3,4-dihydroxyphenylacetic and protocatechuic acids. The distribution of these compounds was similar in both species; however, higher amounts were found in extracts from leaves I. In leaf extracts from *A. arbutifolia*, rosmarinic acid was additionally detected. The amount of this compound in the leaves collected in September (II) was high—154.7 mg/100 g DW.

There have been a few reports on chemical composition of the leaves of *A. melanocarpa* [1, 23, 26]; however, they do not comprehensively characterize this material with respect to phenolic compound content. Teleszko and Wojdyło [26] give only the total amount of phenolic acids, whereas Lee et al. [23] estimated caffeoylquinic acid isomers, seemingly chlorogenic and neochlorogenic acids, but that was not specified. Thi and Hwang [1], on the other hand, found chlorogenic and p-coumaric acids, which were not detected under our study.

According to the analyses performed under this study, the leaves of the three chokeberries are poor in anthocyanins. A very low amount of Cy-Gal (1.2 mg/100 g DW) was estimated only in the leaves of *A. ×prunifolia* (Table 1). Similar results were obtained for the leaves of *A. melanocarpa* by Teleszko and Wojdyło [26]. In the presented work, the comparative analysis of all the groups of phenolic compounds in leaf extracts of *A. ×prunifolia* and *A. arbutifolia* was conducted for the first time.

Based on the obtained results, there was noticeable influence of harvest time on the phenolic composition of leaves of the three *Aronia* species collected at different maturity stages (I and II), The younger leaves, being particularly rich in phenolic acids and flavonols, could be proposed as raw material in the production of high-quality antioxidant functional foods and diet supplements [1, 26, 42]. This use is supported not only by their valuable chemical composition (rich in chlorogenic and neochlorogenic acids) but also by the ease of acquisition, harvesting, and drying [1]. Aronia leaves are also of interest for pharmaceutical and cosmetics industries [43, 44]. In our previous work, we made similar observations concerning the potential use of leaves of *S. chinensis* which could be used alternatively to the more popular fruits of this plant [45, 46].

The chromatographic studies on chokeberry extracts were supported by the analyses of antioxidant capacity of the studied plant material. The estimated antioxidant parameters (FRAP, DPPH, and total phenols) showed significant differences between the fruits of the *Aronia* species involved. Of the examined species, *A. arbutifolia* and *A. melanocarpa* "A" showed the highest activity in DPPH test (30 min), whereas *A. arbutifolia* was demonstrated to be superior in FRAP assay (both 15 and 30 min). These differences were not always in accordance with total phenolics, anthocyanin, flavonoid, and phenolic acid content of the investigated samples. For instance, total phenolic content of *A. melanocarpa* "C" was comparable to that of *A. arbutifolia*, but the former provided significantly lower antioxidant parameters. It has to be noted that the applied measures of antioxidant capacity in vitro are overall assays and include the activity of other constituents of plant matrix, such as thiols, vitamins (especially vitamin C), tannins and their precursors, the nucleotide base-guanine, the trioses-glyceraldehyde and dihydroxyacetone, inorganic ions, and some nitrogen-containing compounds [47]. It is, therefore, difficult to make the direct comparison of the specific results of chromatographic analyses with these measures. The recorded values were similar for all the species studied, thus indicating that the fruits of less known *Aronia* species: *A. ×prunifolia* and *A. arbutifolia* are comparable to *A. melanocarpa* with respect to antioxidant properties.

The fruits of the studied *Aronia* plants showed lower antioxidant potential than leaves of the respective species (Table 4). The highest antioxidant activity was demonstrated for extracts from the leaves of *A. arbutifolia* collected in September (II). For this species, the largest differences in antioxidant parameters between the leaves collected in July (I) and September (II) were also observed. DPPH, FRAP, and total phenols content estimated for the leaves collected in September (II) were about five times higher than for the leaves collected in July (I) (Table 4). Small differences in the measured antioxidant parameters were also observed for the leaves of *A. melanocarpa*. In this case, the leaves from July (I) had lower values of antioxidant parameters than those collected in September (II). The leaves of *A. ×prunifolia* had similar antioxidant parameters regardless of the time of harvesting.

The obtained results of antioxidant capacity clearly indicate that the leaves of the studied *Aronia* sp. should be

taken into consideration when it comes to the preparation of therapeutics or food supplements. The important aspect is that they are readily available throughout almost entire vegetative season and are also suitable for industrial processing. For example, they can be used with relative ease in the production of functional teas.

The presented work demonstrates the importance of comparative analyses of the chemical composition and antioxidant activity of different species of the same genus. Such studies are essential for nominating the species most valuable in terms of biological properties, which can be further employed for the production of functional foods, dietary supplements, and medicines. Similar studies were previously conducted for the representatives of *Rosaceae* family, including different species of the genuses *Rubus* and *Prunus* [48–50].

The present research indicates that fruits and leaves of the less known chokeberries *A. arbutifolia* and *A.* ×*prunifolia* (a hybrid of *A. melanocarpa* and *A. arbutifolia*), can be proposed as valuable medicinal raw materials with high phenolic content and outstanding antioxidant properties. In particular, the study demonstrates that, in this regard, *A.* ×*prunifolia* and *A. arbutifolia* are equivalent to the commonly cultivated *A. melanocarpa*. The work also shows that leaves of the so far underutilized *Aronia* sp. are a rich source of phenolic antioxidants for the use in food supplements and functional food industries. However, further studies including samples collected from different specimens across years are required to determine intra-species variations in phenolic composition and antioxidant properties of the investigated species.

Compliance with ethical standards

Conflict of interest The authors declare that there are no conflicts of interests. All the authors read and approved the manuscript in its final form.

Compliance with ethics requirements This article does not contain any studies with human participants or animals performed by any of the authors.

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